Molecular phylogeny of *Conus chiangi* (Azuma, 1972) (Gastropoda: Conidae)

Maren Watkins
Department of Pathology
University of Utah
Salt Lake City,
Utah, 84112 USA

Patrice Showers Corneli
Department of Biology
University of Utah
Salt Lake City,
UT 84112 USA

David Hillyard
Department of Pathology
University of Utah
Salt Lake City,
UT 84112 USA

Baldomero M. Olivera
Department of Biology
University of Utah
Salt Lake City,
UT 84112 USA

ABSTRACT

Conus chiangi (Azuma, 1972) has been regarded by several workers as morphologically distinctive enough from other *Conus* species to merit placement in its own genus (e.g., *Taranteconus*, Azuma, 1972). We demonstrate using standard molecular markers that this species is related to such well-known species as *Conus imperialis* Linne, 1758, and *Conus regins* Gmelin, 1791, which are generally regarded as belonging to the *Stephanoconus* (Mörch, 1852), clade. *Stephanoconus* has had impressive radiation of species in the new world, but only two species were previously assigned to *Stephanoconus* from the Indo-Pacific region (*Conus imperialis* and *Conus zonatus* Hwass, 1792).

We also present data using a toxinological marker for *Conus chiangi* consistent with its inclusion in *Stephanoconus*. In at least three species of this clade, a virtually identical peptide toxin (first called α-conotoxin Im1 from *Conus imperialis*) was identified. Although the predicted mature peptide toxin sequences are closely similar, significant divergence in the prepro region of the precursors was observed. It is suggested that the conservation of the mature peptide toxin sequence is a result of strong selection related to prey choice in *Stephanoconus*.

The results described here suggest a revised picture of the radiation of *Stephanoconus* in the Indo-Pacific. In addition to the well-known species *Conus imperialis* and *Conus zonatus*, which comprise one group, a radiation of small *Conus* species in deeper water may potentially comprise another distinctive group of *Stephanoconus*.

Additional keywords: Conotoxins, peptides, DNA sequence, molecular markers

INTRODUCTION

Conus chiangi (Azuma, 1972), is a morphologically unusual *Conus* species that differs strikingly from other *Conus* in its spire sculpture (see Figure 1). For many years, *Conus chiangi* was extremely rare and, only very occasionally collected. The type locality is in the South China Sea; a junior synonym of *Conus chiangi* is *Conus lamellatus* (Suzuki, 1972) — the type of *Conus lamellatus* was collected off of Sumisu Island, near Hachijo Island, Izu Peninsula, Japan. Probably because of the unique scale-like spines that line the shoulder margins of the spire, both Azuma and Suzuki proposed that this species defined a new genus: Azuma designated *Taranteconus* as a new genus for this species while Suzuki proposed *Cornutoconus*, reflecting the perceived uniqueness of *Conus chiangi*. The taxonomy of *C. chiangi* has been discussed by Coomans et al. (1983) and Röckel et al. (1995).

More recently, a number of specimens of this unusual cone snail have been collected in the Philippines. Initially, collectors using tangle nets obtained specimens from Cebu and around Bohol Islands. Then, more recently, use of small trawls allowed for collection of an even larger number of specimens off Aliguay Island. Philippine specimens are shown in Figure 1, including an inset illustrating the characteristic spire structure. In this work, we describe a molecular analysis of *Conus chiangi*, using a number of different genetic markers to provide insights into the relationship of *Conus chiangi* to other species of *Conus*. Additionally, we present some unexpected results illustrating selection for conservation of a toxin expressed in *Conus chiangi* venom ducts.

In combination, the data we present below demonstrate that *Conus chiangi* is not phylogenetically distant from other *Conus* species. Thus, there is no justification for the erection of a new genus for this species, given that *Conus chiangi* is shown to be closely related to well-known species such as *Conus imperialis* Linne, 1758, and *Conus regius* Gmelin, 1791, which belong in the *Stephanoconus* clade. This conclusion is reached consistently, no matter which genetic locus we used for the phylogenetic analysis. The more general biological/evolutionary implications of these results are discussed.

MATERIALS AND METHODS

Preparation of Genomic DNA: Genomic DNA was prepared from 20 mg *Conus chiangi* hepatopancreas tissue using the Gentra PUREGENE DNA Isolation Kit Kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's standard protocol.

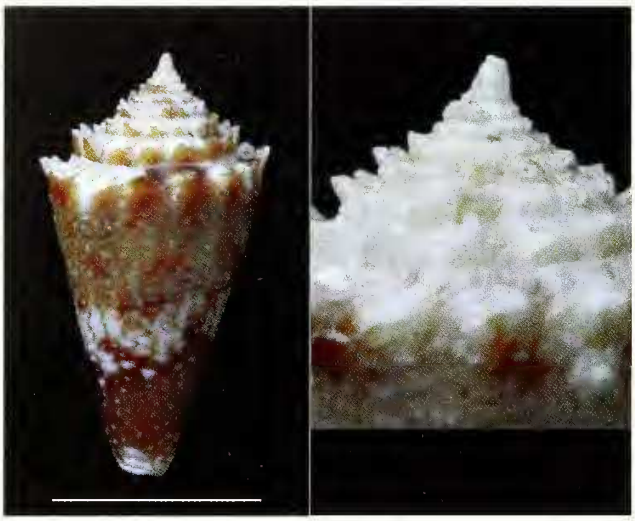

Figure 1. *Conus chiangi* (Azuma, 1972). Left: A specimen of *Conus chiangi*, collected off Aliguay Island, Philippines. Right: The spire of a different specimen of *Conus chiangi*, showing a close-up of the unusual scale-like spine morphology of the spire shoulder margins. Scale bar = 1 cm.

Cloning and Sequencing of 12S and 16 mitochondrial RNA segements and Cytochrome Oxidase subunit I mitochondrial RNA gene segment (BarCOI)

Ten ng of *Conus chiangi* genomic DNA was used as a template for polymerase chain reaction (PCR) with oligonucleotides eorresponding to 12S-I (5′ TCG CAG CAG YCG CGG TTA) and 12S-III (5′ AGA GYG RCG GGC GAT GTG T) mitochondrial rRNA segments and 16SH (5′ CCG GTC TGA ACT CAG ATC ACG T) and 16LC (5′ GTT TAC CAA AAA CAT GGC TTC) mitoehondrial rRNA segments, and oligonueleotides corresponding to COI dgl CO-1490 (5′ GGT CAA CAA ATC ATA AAG AYA TGY G 3′) and COI dgHCO-2198 gene segments (5′ TAA ACT TCA GGG TGA CCA AAR AAY CA 3′). The per cycling profiles are as follows: Initial denaturation (95°C, 60 s); followed by 40 eycles of denaturation (95°C, 20 s); annealing (55°C, 20 s) and extension (72°C, 30 s). The resulting PCR products were purified by gel electropheresis, recovered from agarose using High Pure PCR Product Purifieation Kit (Roehe Diagnostics, Indianapolis, IN). The eluted DNA fragments were annealed to pNEB206A vector using the USER Friendly Cloning kit (New England BioLabs, Inc., Beverly, MA) following manufacturer's suggested protocol and the resulting product transformed into DH5a competent cells. The nucleic aeid sequences of the resulting 12S, 16S and COI-encoding clones were determined according to the standard protocol for Automated sequencing.

Phylogenetic Analysis: Sequences which were first aligned with Clustal X (Larkin et al., 2007) and then refined by eye align obviously homologous regions that Clustal failed to recognize. Individual genes were eoneatenated with MaeClade 4.08 (Maddison and Maddison, 2005).

Sinee MrBayes (Huelsenbeck et al., 2001) has the eapability to eompletely stratify maximum likelihood model parameters by gene and by codon position (Ronquist and Huelsenbeck, 2003), we ehose it as the primary method of tree eonstruetion. Eaeh analysis comprised two simultaneous runs with four chains each. All runs were suffieiently long to minimize the average standard deviation of the split frequeneies (below 0.05 for COI and 0.007 for 12S rRNA and for the eoneatenated sequence of 3 genes. Plots of the number of generations against the maximum likelihood scores indicated apparent equilibrium. Further diagnostics ineluded the potential seale reduction factor (PSRF) that measures the fit of branch length and all parameters. Trees and parameters from the first 25% of the generations were disearded (the burn in) after completion of the MCMC (Monte Carlo Markov Chain) seareh. Bayesian analyses eomprised two runs with four ehains each.

We also used maximum likelihood analysis using models optimized in PHYML (Guindon and Gascuel, 2003). For each tree, we ran a heuristie maximum likelihood seareh followed by analysis of 1000 bootstrap replieates to plaee eonfidenee limits on the trees.

Identification and Sequencing of clones encoding Ch1.I

Genomie DNA from *Conus chiangi*, prepared as deseribed above, was used as a template for polymerase ehain reaction (PCR) with oligonucleotides corresponding to the eonserved intron and 3′ UTR sequences of previously isolated α-eonotoxin genes:

Forward primer: 5′ TGT GTG TGT GTG GTT CTG GGT 3′

Reverse primer: 5′ CTC GAG GTC GTG GTT CAG AGG 3′

The PCR eyeling profiles are as follows: Initial denaturation (95°C, 60 s); followed by 40 eyeles of denaturation (95°C, 20 s); annealing (62°C, 20 s) and extension (72°C, 30 s). The resulting per products were purified using the High Pure PCR Produet Purifieation Kit (Roche Diagnosties, Indianapolis, IN) following the manufaeturer's suggested protocol. The eluted DNA fragments were annealed to pNEB206A veetor, and sequenced as described above.

RESULTS

Molecular phylogeny of *Conus chiangi*: In order to assess the phylogenetie relationships of *Conus chiangi* to other *Conus* species, the sequenees of three standard molecular markers were obtained as described under Materials and Methods above: COI, 12S, and 16S. A tree based on the COI sequences is shown in Figure 2; a set of COI sequences from a much larger number of *Conus* species were initially analyzed. Only a small subset of these results is shown, including the speeies found to be most elosely related to *Conus chiangi*. The data

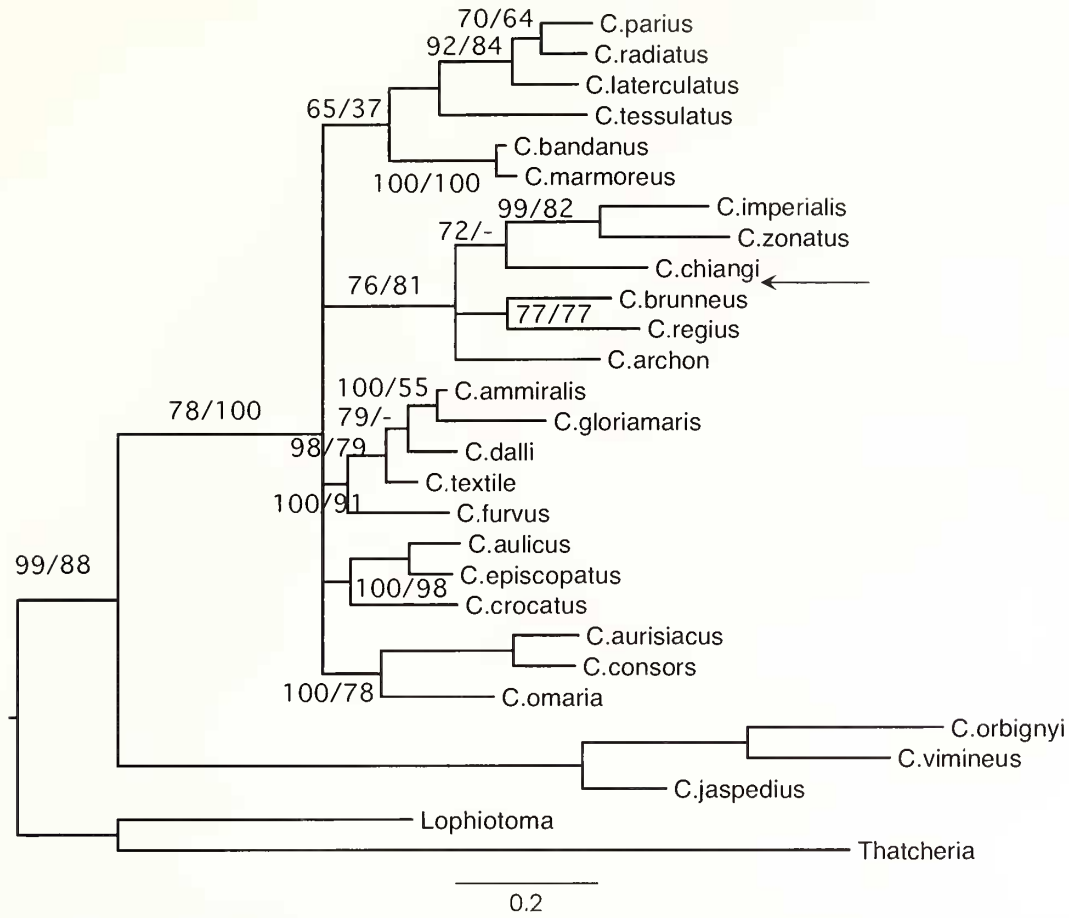

Figure 2. A Bayesian phylogenetic tree based on COI sequences. Numbers to the left of the slash are Bayesian posterior probabilities expressed as pereentages. Numbers to the right are maximum likelihood bootstrap pereentages. Dashes or blanks mark support values less than 50%. These data suggest that *Conus chiangi* is related to *Conus imperialis* and *Conus zonatus*. Sequences of the COI marker of a much larger number of *Conus* species have been obtained, but only a few clades are shown.

presented in Figure 2 suggest that *Conus chiangi* is related to two shallow-water Indo-Pacific species, *Conus imperialis* and *Conus zonatus*. These two species belong to a clade known as *Stephanoconus*, which comprises cone snails known to specialize on devouring amphinomid polychaetes ("fireworms"). There is a greater biodiversity of this group in the new world; in the Indo-Pacific, the only species that are generally assigned to the *Stephanoconus* clade are *Conus imperialis* and *Conus zonatus* (Duda Jr et al., 2001; Espiritu et al., 2001).

The position of *Conus chiangi* was independently assessed using 12SrRNA sequences (Figure 3). These data strongly indicate that *Conus chiangi* is a member of the *Stephanoconus* clade.

We found that an alignment of 16SrRNA, by itself, had too little phylogenetic signal to yield a meaningful tree. However, combining the gene with 12S and COI in a stratified analysis increased the phylogenetic signal to the extent that the relationship of *C. chiangi* with the other *Stephanoconus* species became clear (Figure 4). In this case, there is a 100% posterior probability that *Conus chiangi* belongs within the *Stephanoconus* clade with *Conus regius* and *Conus imperialis*. Furthermore, the data suggests that there are three groups in the clade, one that includes the large Indo-Pacific forms (*Conus imperialis* and *Conus zonatus*), one that includes some of the new world forms (*Conus regius* Gmelin, 1791, and *Conus brunneus* Wood, 1828) and *Conus chiangi*, which in this phylogeny somewhat surprisingly groups together with the Panamic species *Conus archon* Broderip, 1833.

Toxinology: The discovery that *Conus chiangi* venom ducts expressed a certain type of venom peptide (i.e., α-conotoxins (Santos et al., 2004) belonging to the α4/3 subfamily) was an early indication that the species might be a member of the *Stephanoconus* clade (Ellison et al., 2008). Only species in the *Stephanoconus* clade are known to express this unusual group of *Conus* venom peptides, which are targeted to nicotinic acetylcholine receptors. We report here an even more striking

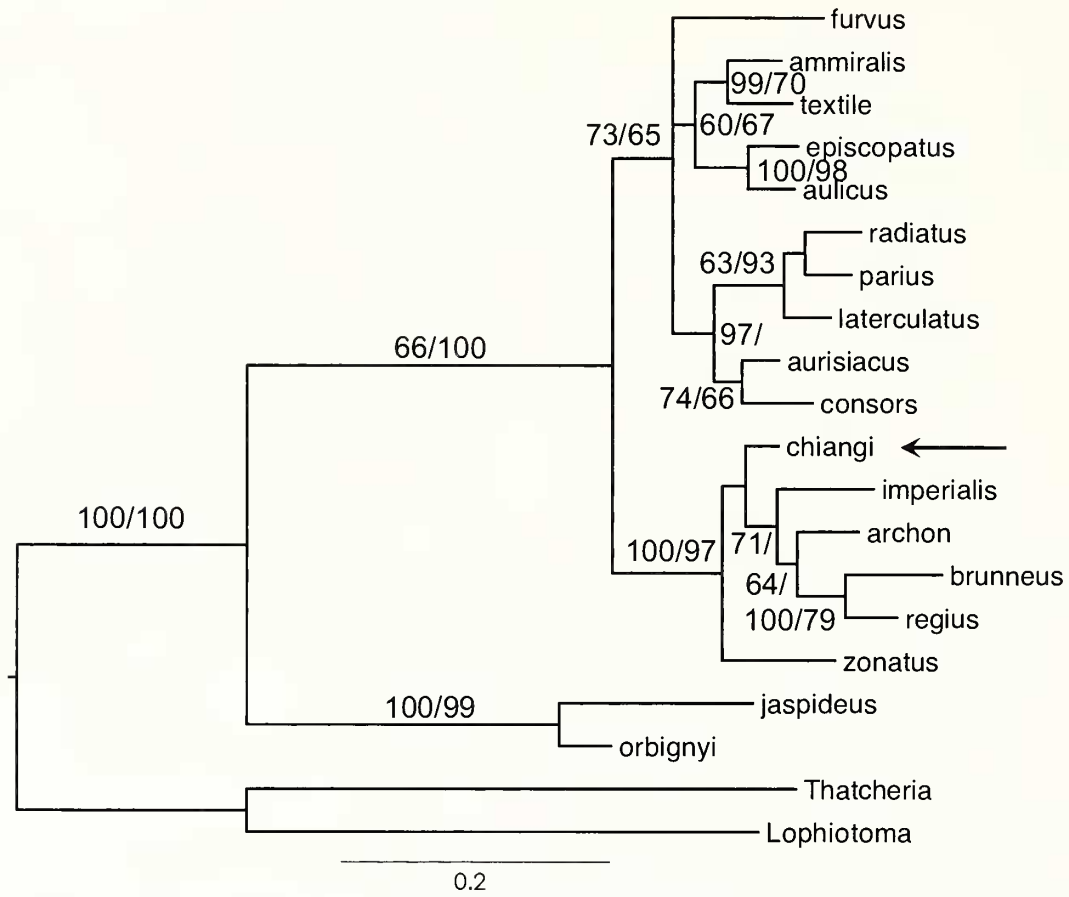

Figure 3. 12SrRNA tree inferred from Bayesian analysis. A topologically identical tree was inferred using maximum likelihood analysis. Branch labels are as described in Fig 2. Note that the clade defined by the bold line (*Stephanoconus*) is well supported using this molecular marker.

illustration of the close affinity of *Conus chiangi* to other species in the *Stephanoconus* clade.

An analysis of peptides in the venom ducts expressed in *Conus chiangi* has revealed a precursor to a peptide previously extensively characterized, α-conotoxin ImI from *Conus imperialis* (McIntosh et al., 1994). In general, there is a striking sequence divergence between *Conus* peptides from different species. The discovery that a peptide identical to α-conotoxin ImI is expressed in the venom duct of *Conus chiangi* provides additional support that the two species are related.

A comparison of precursor sequences predicted from clones obtained from *Conus chiangi, Conus imperialis* and *Conus regius* is shown in Figure 5. The sequences are aligned to maximize sequence identity. Because the predicted precursor sequences for *Conus imperialis* and *Conus regius* were obtained from a genomic clone (see Materials and Methods), these precursor sequences were incomplete but the available sequences have been aligned. The predicted mature peptides are shown in the figure, with the predicted cleavage signals for proteolytic processing underlined.

The sequences shown in the figure reveal that although the mature peptides from *Conus imperialis* and *Conus chiangi* are identical (and the mature peptide from *Conus regius* almost identical); the inferred precursor sequences indicate considerable divergence in the propeptide region. Thus, this is an unprecedented example of identical or almost identical mature peptide sequences with significant differences in the propeptide region of the precursor. The potential significance of these data will be discussed below. However, these results are strong evidence for the close relationship between, and potentially similar biology of, *Conus chiangi, Conus imperalis* and *Conus regius*.

DISCUSSION

The data presented above were obtained from two types of experimental approaches. The phylogentic relationship of *Conus chiangi* to other *Conus* species was first evaluated using three standard marker genes. *Conus chiangi* appears well embedded within the major clade of cone snails (Bandyopadhyay et al., 2008; Puillandre

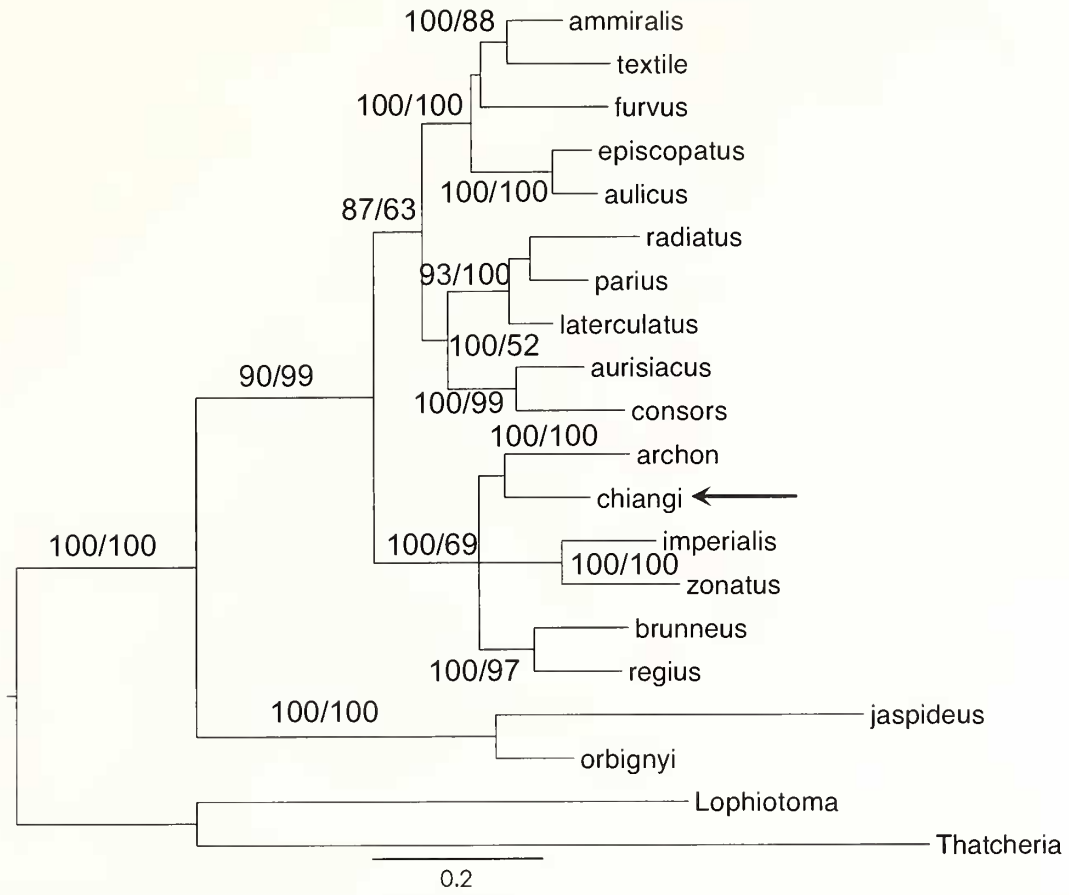

Figure 4. A phylogenetic tree combining all three molecular markers obtained, COI, 12S and 16S) in a stratified Bayesian analysis. An identical maximum likehood tree supports this topology. Labels are as in Figure 2. In this case, the assignment of *Conus chiangi* to the *Stephanoconus* clade (type of the proposed subgenus *Stephanoconus* is *Conus regius*) is well supported.

Sequences from genomic clones:

Ch1.1	...FDGRNAAADDKASDLIAQIVRRGCCSDPRCAWRC
α-Iml	...LEERNAPADDKASDLIAQIVRRGCCSDPRCAWRC
Rg1.9	...FNGRSAAADQNAPGLIAQVVRGGCCSDPRCAWRC

Predicted mature α-conotoxin sequence:

Clone	Species	Predicted toxin sequence
Ch 1.1	*Conus chiangi*	GCCSPDRCAWRC#
αIml	*Conus imperialis*	GCCSPDRCAWRC#
Rg1.9	*Conus regius*	GGCCSPDRCAWRC#

Figure 5. Similarity of mature α-conotoxin sequences of *Conus chiangi* with those from *Conus imperialis* and *Conus regius*. Shown are data obtained from genomic clones from *Conus chiangi*, *Conus imperialis* and *Conus regius*. The predicted proteolytic processing site following which cleavage occurs to release the mature toxin is underlined. Note that the mature toxin sequences are virtually identical in the three species despite considerable divergence in the propeptide region. # - denotes an amidated C-terminus

et al., 2008) and is most closely related to *Conus* species traditionally assigned to the *Stephanoconus* clade.

The second type of data described above were toxinological markers; these are also consistent with the assignment of *Conus chiangi* to the *Stephanoconus* clade. The discovery of identical or almost identical peptide sequences from the venom ducts of *Conus chiangi*, *Conus imperialis* and *Conus regius* establishes the close relationship between the three species despite the strikingly divergent shell morphology of *Conus chiangi*. In contrast to the other two shallow-water species, it is only found in relatively deep water (200–300 meters). This is the first documentation of almost identical peptides from three different *Conus* species—even peptides that seem to share the same molecular targeting specificity can vary greatly in their primary amino acid sequence if closely related *Conus* species are compared. α-Conotoxin ImI has been an extensively characterized competitive antagonist of nicotinic acetylcholine receptors. In the mammalian nervous system, this peptide competitively antagonizes a

Figure 6. Shells of some species in the *Stephanoconus* elade. All speeies in the *Stephanoconus* clade that are ineluded in the analysis in Figure 4 are shown. Top, left to right: *Conus chiangi, C. imperialis, C. zonatus;* bottom, left to right: *C. regius, C. arcbon, C. brunneus*. The specimen of *Conus brunneus* shown is a juvenile (when adult, it is approximately the same size as the specimen of *Conus regius*). All of the other specimens, ineluding *C. chiangi* are full-sized adults. Note how mueh smaller *Conus chiangi* is than the other species in this clade. Scale bar = 1 cm, applies to all images.

variety of neuronal subtypes, including $\alpha 7$ and $\alpha 3\beta 2$ nicotinic receptors. Injection of the peptide into the central nervous system of a mouse elieits partial seizures.

Potentially more relevant to the biology of the eone snails is the observation that this peptide also inhibits one of the nicotinic acetylcholine receptors at the neuromuscular junetion of the nematode *Caenorhabditis*

elegans (J. Richmond and E. Jorgensen, unpublished results). Thus, the peptide appears to be active over a broad phylogenetic range, although it seems to be highly selective for a specific subset of nicotinic acetylcholine receptors in an individual nervous system. In invertebrates, it may well be targeted to nicotinic receptors that are important for neuromuscular transmission, and thus may be a major venom component that enables polychaete-hunting cone snails to cause prey paralysis.

The unusual conservation of the mature toxin region may be a consequence of the small size of this peptide. There may be stringent selection on the sequence of the peptide. There is some direct evidence to support this: native peptides that are closely homologous in sequence, such as α-conotoxin ImII and α-conotoxin RgIA, have different molecular targeting specificity (for a review, see Olivera et al., 2008). Thus, the mature α-conotoxin ImI peptide, which is also found in *Conus ehiangi* venom, has presumably been optimized by strong selection, and given the small size, does not show the typical hypervariability that can be demonstrated in larger homologous peptides from different *Conus*. Nevertheless, that the genes involved are subject to hypervariation is demonstrated by the lack of conservation in the propeptide region between the three species compared in Figure 5.

Thus, despite highly divergent morphological characteristics of the shell of *Conus ehiangi*, it is clearly related to other *Conus* and there would appear to be no justification for separating it into a separate genus. The assignment of *Conus chiangi* to the genus *Conus* (and subgenus *Stephanoconus*) would be consistent with the results presented above. [It should be noted that some workers (see for example da Motta, 1991) do not assign *C. imperialis* to *Stephanoconus*, but to *Lithoconus* Morch, 1852. Since the type of *Lithoconus* is *C. leopardus*, which is not closely related to *C. imperialis* by molecular phylogeny, the assignment to *Stephanoconus* seems more appropriate].

A curious feature of the *Stephanoconus* clade (see Figure 6 for some examples) is that there were relatively few species assigned to this clade in the Indo-Pacific, with greater biodiversity in the Caribbean/Eastern Pacific. Although not all candidates for assignment to the *Stephanoconus* clade have been directly analyzed using molecular markers, it seems likely that *Conus brunneus* Wood, 1828, *Conus bartschi* Hanna and Strong, 1949, and *Conus archon* Broderip, 1833, in the Eastern Pacific, and *Conus regius* Gmelin, 1791, *Conus aurantius* Hwass, 1792, and the complex of forms related to *Conus cedonulli* Linne, 1767 in the Caribbean, are members of the *Stephanoconus* clade. Although the Indo-Pacific has much greater biodiversity of *Conus* species in general, only two species have routinely been assigned to this clade, the widely distributed *Conus imperialis* Linne, 1758, and *Conus zonatus* Hwass, 1792, found in the Indian Ocean from South India to Western Thailand. The results of our study, which has revealed that *Conus ehiangi* is well embedded in the *Stephanoconus* clade, raises the possibility that there is a deep-water Indo-Pacific radiation of *Stephanoconus*. It would seem useful to investigate whether other *Conus* species with unusual spire structures belong to this group, including two other small deep-water species, *Conus suidurati* and *Conus polongimarumai* Kosuge, 1980 (see Figure 7). From similarities in shell morphology, the latter seems particularly likely to be a deep-water *Stephanoconus* like *Conus ehiangi*.

The discovery that *Conus ehiangi* belongs to the *Stephanoconus* clade has biological implications: all

Figure 7. *Conus chiangi* compared to other putative members of a deep-water *Stephanoconus* clade. The two left-most specimens are *Conus chiangi*; the two middle specimens are *Conus polongimarumai* and the right-most specimen is *Conus suidirati*. The two species *Conus polongimarumai* and *Conus suidirati* are rare, and no molecular data has been obtained. Based on morphological criteria, these are candidates for inclusion in a deep-water Indo-Pacific clade of *Stephanoconus*, defined by *Conus chiangi*. Scale bar = 1 cm, applies to all images.

known members of the *Stephanoconus* clade whose prey preference has been defined eat amphinomid polychaetes ("fireworms") (Kohn, 1959; Röckel et al., 1995). The molecular phylogeny of *Conus chiangi*, and the conservation of venom peptides documented above, suggest that the prey preference of the species is related: likely small, deep-water amphinomids. Whether or not this prediction is true can be verified by a type of reverse ecological approach that was recently used for identifying the prey of some turrid species (M. Astilla and G. Concepcion, unpublished) in which a PCR analysis of the gut contents of several Turrid species (to identify the barcode sequence of recently ingested prey) was used. However, the tree in Figure 4 suggests that the deep water Indo-Pacific *Stephanoconus* diverged from the two shallow water species relatively early in the adaptive radiation of the clade, comparable in time to when the new world and old world branches diverged.

The *Stephanoconus* clade, as redefined here, has several intriguing features. It is one of the few worldwide clades within the genus *Conus*. In contrast to *Stephanoconus*, the *Conus* species that prey on fish display an entirely different biogeographic pattern. The available molecular data indicate that the new world piscivorous species evolved fish-hunting completely independently, and are not genetically more closely related to fish-hunting than to non fish-hunting *Conus* clades (Duda Jr. and Palumbi, 2004; Espiritu et al., 2001; Imperial et al., 2007) (Krause et. al., manuscript in preparation). The fact that *Stephanoconus* is both worldwide in its distribution, and significantly more species-rich in the new world, is different from most other characterized branches of *Conus*, suggesting an unusual evolutionary history of this group compared to other cone snail clades.

ACKNOWLEDGMENTS

This work was supported by a program project grant (GM48677) from the National Institute of General Medical Sciences. We are grateful to Kerry Matz and Tuong Huynh for preparing some of the figures.

LITERATURE CITED

Azuma, M., 1972. Descriptions of Four New Gastropods from South China Sea. Venus 31: 56–61.

Bandyopadhyay, P.K., B.J. Stevenson, J.P. Ownby, M.T. Cady, M. Watkins, and B.M. Olivera. 2008. The mitochondrial genome of Conus textile, coxI-coxII intergenic sequences and Conoidean evolution. Molecular and Phylogenetics Evolution 46, 215–23.

Coomans, H.E., R.G. Moolenbeek, and E. Wils. 1983. Alphabetical revision of the (sub) species ub recent Conidae 7. *cingulatus* to *cylindraceus*, including *Conus shikamai nomen novum*. Basteria 48: 223–311.

da Motta, A.J. 1991. A systematic classification of the gastropod family Conidae at the generic level. La Conchiglia, Rome.

Duda Jr, T.E. and S.R. Palumbi. 2004. Gene expression and feeding ecology: evolution of piscivory in the venomous gastropod genus *Conus*. Proceedings of the Royal Society London 271: 1165–1174.

Duda Jr, T.F., A.J. Kohn, and S.R. Palumbi. 2001. Origins of diverse feeding ecologies within *Conus*, a genus of venomous marine gastropods. Biological Journal of the Linnean Society 73: 391–409.

Ellison, M., Z.P. Feng, A.J. Park, X. Zhang, B.M. Olivera, J.M. McIntosh, and R.S. Norton. 2008. Alpha-RgIA, a novel conotoxin that blocks the alpha9alpha10 nAChR: structure and identification of key receptor-binding residues. Journal of Molecular Biology 377: 1216–1227.

Espiritu, D.J.D., M. Watkins, V. Dia-Monje, G.E. Cartier, L.J. Cruz, and B.M. Olivera. 2001. Venomous cone snails: molecular phylogeny and the generation of toxin diversity. Toxicon 39: 1899–1916.

Guindon, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.

Huelsenbeck, J.P., F. Ronquist, R. Nielsen, J.P. and Bollback. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310–2314.

Imperial, J., N. Silverton, B.M. Olivera, P. Bandyopadhyay, A. Sporning, M. Ferber, and H. Terlau. 2007. Using chemistry to reconstruct evolution : On the origins of fish-hunting in venomous cone snails. Proceedings of the American Philosophical Society 151: 185–200.

Kohn, A.J. 1959. The Ecology of *Conus* in Hawaii. Ecology Monograph 29: 47–90.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, and D.G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.

Maddison, D.R., and W.P. Maddison. 2005. MacClade 4.08.

McIntosh, J.M., D. Yoshikami, E. Mahe, D.B. Nielsen, J.E. Rivier, W.R. Gray, and B.M. Olivera. 1994. A nicotinic acetylcholine receptor ligand of unique specificity, a-conotoxin Iml. Journal of Biological Chemistry 269: 16733–16739.

Olivera, B.M., M. Quik, M. Vincler, and J.M. McIntosh. 2008. Subtype-selective conopeptides targeted to nicotinic receptors: Concerted discovery and biomedical applications. Channels 2: 143–152.

Puillandre, N., S. Samadi, M.C. Boisselier, A.V. Sysoev, Yu.I. Kantor, C. Cruaud, A. Couloux, and P. Bouchet. 2008. Starting to unravel the toxoglossan knot: molecular phylogeny of the "turrids" (Neogastropoda, Conoidea). Molecular Phylogenetics and Evolution 47: 1122–1134.

Röckel, D., W. Korn, and A.J. Kohn. 1995. Manual of the Living Conidae. Verlag Christa Hemmen, Wiesbaden, 358 pp.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Santos, A.D., J.M. McIntosh, D.R. Hillyard, L.J. Cruz, and B.M. Olivera. 2004. The A-superfamily of conotoxins: structural and functional divergence. Journal of Biological Chemistry 279: 17596–17606.

Suzuki, M., 1972. Descriptions of two new species of gastropods. Pacific Shell News 5: 4 pp. [unnumbered]